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Indian Standard

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बासमती चावल की प्रजाति पहचान के लिए माइक्रोसेटेलाईट आधारित पी सी आर विश्लेषण पद्धति

Microsatellite Based PCR Protocol for Identification of Basmati Rice Varieties

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Biotechnology for Food and Agriculture Sectional Committee, FAD 23

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards after the draft finalized by the Biotechnology for Food and Agriculture Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Basmati is long grain aromatic rice grown for centuries in the Indo-Gangetic plains of Himalayan foot hills, characterized by extra-long slender grains that elongate approximately twice of their original length on cooking with soft and fluffy texture, delicious taste, pleasant aroma and distinct mouth feel. The primary and ancillary characters of Basmati rice as defined in the Memorandum of Ministry of Agriculture, Government of India (No. 17-12/2007-SD.IV dated 29th May 2008) are listed in the Annex A of this standard. In addition to genetic make-up, agro-climatic conditions of the specific geographical area as well as method of harvesting, processing and aging are crucial for the best expression of the aforesaid attributes of Basmati rice. The Government of India has protected Basmati rice under the Geographical Indication (GI) comprising of entire states of Punjab, Haryana, Delhi Himachal Pradesh, Uttarakhand and parts of Uttar Pradesh and Jammu and Kashmir (*see* Annex A). In India, as of now, there are 32 Basmati rice varieties notified under the *Seeds Act*, 1966 (*see* Annex A).

India is a leading exporter of the Basmati rice with annual export of nearly 4 million tonnes with forex earning of Rs. 25,000 crores (US$ 3.62 billion) currently. In order to ensure the authentic quality of Basmati rice to consumers, DNA protocol for authentication and admixture detection was developed by Centre for DNA Fingerprinting & Diagnostics (CDFD), Hyderabad using microsatellite marker based analytical method. The protocol presented here, describes DNA extraction, PCR and genotyping procedure for authentication of 30 notified Basmati rice varieties and quantification of admixtures, if any. The process of notification of new varieties of Basmati rice under the *Seeds Act*, 1966 is dynamic. Therefore, the standard may need revision every two-three years.

In the preparation of this standard, due consideration has been given to the *Food Safety and Standards Act*, 2006 and Rules framed thereunder. The standard is however subject to restrictions imposed under these rules wherever applicable.

Annex A of this standard is for information only.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

MICROSATELLITE BASED PCR PROTOCOL FOR IDENTIFICATION OF BASMATI RICE VARIETIES

1 SCOPE

This standard prescribes the method using specific nucleic acid analysis for identification of Basmati rice varieties and for subsequent detection of adulteration in Basmati rice, intended to be used by laboratories involved in identification of Basmati rice varieties.

NOTE — The method given in this standard can quantify the percent level of admixture of the non-Basmati rice varieties referred in this standard that are commonly used for adulteration of the basmati rice varieties.

2 REFERENCES

The following standard contains provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated was valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

IS No.	*Title*
16578 : 2018	Foodstuffs — Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Basmati Rice — Basmati rice (*Oryza sativa*) shall mean Basmati raw milled rice; parboiled rice; husked and unpolished (brown) Basmati rice; and parboiled brown Basmati rice grown in the Indo-Gangetic plains having exceptional length of grain, which increases substantially on cooking and the cooked grain has high integrity and high discreteness; and distinctive aroma, taste and mouth feel. All varieties of rice notified as 'Basmati' under *Seeds Act*, 1966 by Government of India shall be termed as Basmati rice.

3.2 Terms Related to Nucleic Acid Extraction and Purification

3.2.1 *Nucleic Acid Extraction* — Sample treatment for the liberation of target nucleic acid.

NOTE — The nucleic acid extraction procedure is used for isolating nucleic acids from other cellular components, such as protein, lipids, carbohydrates and other impurities in a test sample.

3.2.2 *Nucleic Acid Purification* — Method resulting in a more purified DNA.

NOTES:

1 A procedure or process involving sequential steps used to separate DNA and/or RNA from other components in a sample. A highly purified DNA or RNA sample contains negligible observable or measurable effects attributable to inhibitors of the polymerase chain reaction (PCR).

2 In this context, purity refers to the reduction of observable and measurable effects of PCR inhibitors.

3.3 Terms Related to PCR Amplification of Nucleic Acids

3.3.1 *Amplicon* — Specific DNA fragment produced by PCR.

3.3.2 *Hybridization* — Specific binding of complementary nucleic acid sequences under suitable reaction conditions.

3.3.3 *Multiplex PCR* — PCR that uses multiple pairs of primers targeting different loci combined within a single reaction mixture to produce multiple amplicons simultaneously.

3.3.4 *Polymerase Chain Reaction (PCR)* — Enzymatic procedure which allows *in-vitro* amplification of DNA.

Microsatellites occur at thousands of locations within an organism's genome and have a higher mutation rate than other areas of DNA leading to high genetic diversity.

3.3.5 *Primer* — Oligonucleotide of defined length and sequence complementary to a segment of an analytically relevant DNA sequence.

3.3.6 *Probe* — Labelled nucleic acid molecule with a defined sequence used to detect target DNA by hybridization.

3.3.7 *Specificity* — Property of a method to respond exclusively to the characteristic or analyte under investigation.

NOTE — It describes the ability to specifically recognize the nucleic acid sequence to be detected by distinguishing it from other nucleic acid sequences, and the tendency for a primer or probe to hybridize with its intended target and not hybridize with other non-target sequences.

3.3.8 *Thermocycler* — Automated laboratory apparatus used to repeatedly raise and lower the temperature of a sample by cycling through a series of discrete, pre-programmed steps.

NOTE — This cycling of temperatures drives the PCR process.

3.4 Term Related to Detection

3.4.1 *Electrophoresis* — Method of separating electrically-charged particles by their differential migration under an electric field.

NOTE — PCR products can be separated by various types of electrophoresis.

3.5 Terms Related to Controls

3.5.1 *Reference Sample/Reference Material* — Material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

NOTE — The reference material may either be provided by the customer, internal to the laboratory, or an officially-designated reference.

3.5.2 *Test Control* — One or more samples that have undergone all or part of the analytical process designed for the target samples and which can reveal known alleles of the markers used, thereby signalling any process errors and providing reference alleles which can facilitate the reading of results.

3.6 Terms Related to Markers

3.6.1 *Allele Competition* — Preferential amplification of one allele over another in a heterozygote or a mixture.

3.6.2 *Allele Frequency* — Measure of how common an allele is in a population; the proportion or percentage of all of the occurrences of a locus that is occupied by a given allele.

3.6.3 *Genotyping* — Process of determination of genetic variants of an organism.

3.6.4 *Marker* — Genetic marker that typically applies to DNA fragments matching a given locus that gives information on the genotype of the carrier or on the genotype of neighbouring loci.

3.6.5 *Microsatellite or Simple Sequence Repeat (SSR)* — It is a region of DNA consisting of a short (1 bp to 6 bp) sequence (repeat unit) that is tandemly repeated many (typically five to 50) times.

NOTES

1 SSRs are commonly known as microsatellites.

2 The number of repeat units present at a specified SSR, and thus the overall length of the SSR, often varies among individuals.

3.6.6 *Null Allele* — PCR sequence variant that precludes PCR amplification of a particular target, resulting in the absence of detectable PCR product.

3.6.7 *Repeat Region* — Genomic region in which a particular DNA or RNA sequence occurs as multiple copies.

3.6.8 *Single Nucleotide Polymorphism (SNP)* — SNP is the single nucleotide variation in a genetic sequence that occurs at appreciable frequency in the population.

NOTE — SNP is often pronounced as “snip”.

4 PRINCIPLES

4.1 This standard prescribes a procedure for a ‘single-tube assay’ to generate Basmati rice variety-specific allele profiles, whereby PCR reactions are multiplexed by suitably combining in single tube primers for ten SSR loci so that the resulting amplicon sizes do not overlap with each other. This method can be used for the unambiguous identification of different Basmati rice varieties. However, for quantitative detection of adulteration in Basmati rice samples, the conventional singleplex PCR is suggested where a single SSR locus is amplified in a single reaction tube.

4.2 Microsatellites, also known as simple sequence repeats (SSRs), are simple tandemly repeated di- to penta-nucleotide sequence motifs flanked by unique sequences. Their abundance and hyper variability make them valuable as genetic markers. The assay for SSR analysis comprises the following steps:

a) Sample preparation;
b) DNA extraction;
c) PCR amplification; and
d) Genotyping, that is, electrophoretic separation and visualization of the PCR products.

5 APPARATUS

5.1 Mixer Grinder

5.2 Plant DNA Isolation Kit

5.3 96-well Microplate

5.4 Vortex

5.5 Microplate Centrifuge

5.6 Adjustable-volume Micropipettes

5.7 Thermocycler

5.8 Genetic Analyzer

5.9 Refrigerated Centrifuge

6 REAGENTS

6.1 PCR Reagents

6.1.1 *DNA Polymerase*

6.1.2 *Buffer*

6.1.3 *Magnesium Chloride (MgCl2)*

6.1.4 *Deoxyribonucleotide Triphosphates* (dNTPs)

6.1.5 *Primers*

6.2 Genotyping Reagents

6.2.1 *Separation Matrix or Polymer (POP or Equivalent)*

6.2.2 *Running Buffer*

6.2.3 *Reference Dye (Dye ROX 500 or Equivalent)*

6.2.4 *Highly Deionized Formamide* (*Hi-Di formamide or equivalent*)

7 PROCEDURES

7.1 Sample Preparation

50 g of rice sample is ground to a fine powder in a suitable mill or grinder. Grinding is done at pulsed time intervals so that the sample temperature does not exceed 60ºC.

7.2 DNA Extraction

Genomic DNA from 100 mg of the powdered sample is extracted using a plant DNA isolation kit following the manufacturer's protocol.

7.3 PCR Amplification

The conditions shall be set as optimized for the PCR thermal cycler.

7.3.1 Mixture preparation for multiplex PCR shall be as per Table 1.

7.3.2 Mixture preparation for singleplex PCR shall be as per Table 2.

7.3.3 Amplification conditions for both multiplex and singleplex shall be as per Table 3.

Table 1 Mixture Preparation for Multiplex PCR (Final PCR Volume = 10 µl)

(*Clauses* 7.3.1 *and* 11.1)

Sl No.	Stock Solution	Final Concentration/ Amount	Volume for 1 ×
i)	PCR grade water	-	1.1 µl
ii)	Buffer 10 × (100 mM Tris-HCl, pH 8.3, 500 mM KCl)	1X	1 µl
iii)	dNTPs (1 mM)	0.1 mM	1 µl
iv)	$MgCl_2$ (25 mM)	2 mM	0.8 µl
v)	DNA Taq polymerase (5U/µl)	0.5 U	0.1 µl
vi)	10 forward primers as per Table 5 (each 5 µM, fluorophore-labelled)	0.1 µM	0.2 µl each × 10 = 2 µl
vii)	10 reverse primers as per Table 5 (each 5 µM, fluorophore-labelled)	0.1 µM	0.2 µl each × 10 = 2 µl
viii)	DNA template (15 ng/µl)	30 ng	2 µl

Table 2 Mixture Preparation for Singleplex PCR (Final PCR Volume = 10 µl)

(*Clause* 7.3.2)

Sl No.	Stock Solution	Final Concentration/ Amount	Volume for 1X
i)	PCR grade water	-	4.7 µl
ii)	Buffer 10X (100 mM Tris-HCl, pH 8.3, 500 mM KCl)	1X	1 µl
iii)	dNTPs (1mM)	0.1 mM	1 µl
iv)	$MgCl_2$ (25mM)	2 mM	0.8 µl
v)	DNA Taq polymerase (5U/µl)	0.5 U	0.1 µl
vi)	Single forward primers as per Table 5 (each 5 µM, fluorophore-labelled)	0.1 µM	0.2 µl
vii)	Single reverse primers as per Table 5 (each 5 µM, fluorophore labelled)	0.1 µM	0.2 µl
viii)	DNA template (15 ng/ µl)	30 ng	2 µl

NOTE — In preparing the mixture for PCR reactions in Table 1 and 2, reagents from Sl No. *(i)* to *(vii)* are pre-mixed in appropriate multiples of the indicated volumes to prepare a "1X Master mix", 8µl of which is then mixed with 2 µl of DNA template at Sl. No. *(viii)* for each multiplex PCR reaction.

Table 3 Amplification Conditions

(*Clauses* 7.3.3 *and* 11.1)

Initial Denaturation	Denature	Anneal	Extend	Final Extension	Hold
	25 cycles ⟷				
94°C for 10 min	94°C for 45 s	55°C for 90 s	72°C for 60 s	60°C for 30 min	4°C

7.4 Genotyping as Optimized on Capillary Electrophoresis System

7.4.1 Calibrate the instrument and prepare for the electrophoresis as per the manufacturer's guidelines.

7.4.2 Prepare denaturing solution in units of volume given in Table 4 multiplied by the total number of PCR reactions to be analyzed.

Table 4 Unit Volumes for Preparation of Denaturing Solution

(*Clause* 7.4.2)

Component	Volume for 1 reaction (in µl)
Reference dye	0.3
Highly deionized formamide	12

7.4.3 Add 12.3 µl of denaturing solution to each well of the microplate followed by 0.2 µl of PCR product. If there are fewer samples than that required for a full-capacity run, the remaining wells are filled with 10 µl highly deionized formamide and marked as 'empty' in the plate record.

7.4.4 Place a clean, dry, grey septa cover over the loaded microplate and spin down in a microplate centrifuge.

7.4.5 Denature the samples by placing the microplate in a thermal cycler at 95°C for 5 min chill on ice for 3 min.

7.4.6 Load the plate onto the genetic analyzer and subject the samples to capillary electrophoresis by selecting, the appropriate run module of the software for fragment analysis as mentioned in the instrument user manual.

7.4.7 Perform data analysis using software as provided with the instrument.

8 PRIMER SEQUENCES AND MICROSATELLITE LOCI EMPLOYED FOR MULTIPLEX/SINGLEPLEX PCR ASSAY

Primer sequences for the ten SSR loci, and corresponding PCR amplicon sizes of different alleles, are given in Table 5 (sequence source for the microsatellite markers: www.gramene.org).

9 INTERPRETATIONS AND EXPRESSION OF RESULTS

9.1 Specific Allele Profiles of Rice Varieties

Allele sizes of different SSR loci for 30 different Basmati rice varieties and certain non-Basmati varieties are given in Table 6.

Table 5 Characteristics of the Microsatellite Loci and Primers for Multiplex/Singleplex PCR

(*Clause* 8, Table 1 *and* Table 2)

Sl No. (1)	SSR Locus (2)	Repeat Motif (3)	Chromo some (4)	Forward Primer (5’ to 3’) (5)	Reverse Primer (5’ to 3’) (6)	Allele Sizes in Base Pairs (7)	Fluorophore (5’ End of Forward Primer Tagged) (8)
i)	RM171	$(GATG)_n$	10	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	322 335 343 348	FAM
ii)	RM55	$(GA)_n$	3	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	218 220 228 233 237	TAMRA
iii)	RM202	$(GA)_n$	11	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA	162 182 186	JOE
iv)	RM72	$(TAT)_nCT(ATT)_{3n}$	8	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	150 159 164 174	FAM
v)	RM348	$(CAG)_n$	4	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC	131 140	TAMRA
vi)	RM241	$(GA)_n$	4	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG	130 141 147	JOE
vii)	RM44	$(GA)_n$	8	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	103 108 112	TAMRA
viii)	RM1	$(GA)_n$	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	74 101 106 109	FAM
ix)	RM228	$CA_6(GA)_{36}$	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	106 108 115 117 145 153	JOE
x)	RM11997	$(AAT)_{40}$	1	TGTTGTACCAGATGCCCATGTACC	CCGCGTTCACATAACGATGC	582 626 645	FAM

5’-FAM = 5’-carboxyfluorescein; TAMRA = carboxytetramethyl rhodamine; JOE = 6-carboxy-4’-5’-dichloro-2’-7’-dimethoxyfluorescein.

Table 6 Rice variety-specific allele sizes at the different SSR loci

(*Clauses* 9.1, 10 *and* 11)

Sl No.	Rice Variety	SSR MARKERS									
		RM171	**RM55**	**RM202**	**RM72**	**RM348**	**RM241**	**RM44**	**RM1**	**RM 228**	**RM 11997**
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
	Basmati varieties										
i)	Basmati 217	343	228	182	159	140	130	103	101	-	-
ii)	Basmati 370	335	233	182	174	140	141	108	74	115	626
iii)	Type-3 (Dehraduni Basmati)	335	233	182	174	140	141	108	74	115	626
iv)	Ranbir Basmati	335	233	182	174	140	147	108	74	117	626
v)	Taraori Basmati	335	218	182	174	140	130	112	74	106	626
vi)	Basmati 386	335	218	182	174	140	130	112	74	106	626
vii)	Punjab Basmati 1	343	220	162	150	140	141	103	74	-	-
viii)	Pusa Basmati-1	343	228	182	159	131	130	112	74	108	582
ix)	Kasturi	343	228	182	159	131	141	103	74	108	582
x)	Mahi Sugandha	343	228	162	150	131	130	103	101	115	582
xi)	Haryana Basmati	343	228	162	159	131	130	103	109	106	582
xii)	Improved Pusa Basmati 1 (Pusa 1460)	343	228	182	159	131	130	112	74	108	582
xiii)	Pusa Basmati 1121	335	218	182	174	131	130	112	74	108	626
xiv)	Vallabh Basmati 22	335	228	182	159	131	130	112	75	108	582
xv)	Pusa Basmati 6 (Pusa 1401)	343	228	182	174	131	130	112	74	108	582
xvi)	Punjab Basmati 2	343	218	182	174	140,131	130	108	74	106	582
xvii)	Basmati CSR 30	335	218	182	174	140,131	130	112	74	108	626
xviii)	Malviya Basmati Dhan	322	228	182	159	131	130	103	106	106	582
xix)	Vallabh Basmati 21	322	233	186	159	131	141	103	109	106	582
xx)	Pusa Basmati 1509	343	218	182	174	131	130	112	74	145	582
xxi)	Vallabh Basmati 23	335	233	182	159	140,131	130	112	74	108	582
xxii)	Vallabh Basmati 24	343	228	162,182	159,174	131	130	103	74	108	582
xxiii)	Basmati 564	335	233	162	174	140	141	113	101	115	582
xxiv)	Pusa Basmati 1609	343	228	182	159	131	130	103	106	145	582
xxv)	Pant Basmati 1	322	228	162	159	140,131	130	103	74	106	582
xxvi)	Pant Basmati 2	322	228	182	150	131	130	112	106	106	582
xxvii)	Punjab Basmati 3	335	218	182	174	140,131	130	112	74	106	582
xxviii)	Pusa Basmati 1637	343	228	182	159	131	130	112	74	108	582
xxix)	Pusa Basmati 1728	343	228	182	174	131	130	112	74	108	582
xxx)	Pusa Basmati 1718	335	218	182	174	131	130	112	74	108	626
	Non-Basmati varieties (Adulterants)*										
xxxi)	Sharbati	322	228	162	159	131	130	103	106	106	645
xxxii)	IR 64	348	228	186	164	131	130	103	74	153	582
xxxiii)	Jaya	322	237	162	159	131	130	103	106	106	582
xxxiv)	Pusa Sugandh 2	343	228	162	159	131	130	103	106	145	582
xxxv)	Pusa Sugandh 3	343	228	162	159	131	130	103	106	145	582
xxxvi)	Pusa Sugandh 5	343	228	182	159	131	130	103	106	145	582

* The list of non-Basmati rice varieties listed as adulterants is indicative.

10 INTRA-LABORATORY VALIDATION STUDIES: STABILITY OF MARKER PROFILES

The test method given in this standard was developed through genotyping undertaken for at least ten individual plants from each of the rice varieties listed in Table 6. There was no within-variety variation at any of the marker loci listed above. Varietal homozygosity and lack of intra-varietal variation among the rice varieties, coupled with moderate allele frequency of the selected microsatellite loci, ensure stability of the genotyping assay as described here.

11 OPTIONAL METHOD FOR IDENTIFYING RESISTANT BASMATI VARIETIES

In the recent past, a number of Basmati rice varieties such as Pusa Basmati 1637, Pusa Basmati 1718 and Pusa Basmati 1728, have been developed through marker assisted backcross breeding for improvement of resistance to diseases in popular Basmati rice varieties such as Pusa Basmati 1, Pusa Basmati 1121 and Pusa Basmati 6, respectively. These varieties, do not differ from their respective recurrent parents with respect to the profile of the 10 SSR markers listed in Table 6. Since, these varieties are developed by incorporating specific genes for resistance to bacterial blight (Xa13 and Xa21) and blast (Piz 5, Pi 54 and Pi9), the gene linked/gene-based markers as given in Table 7 can be readily used for the purpose of their distinction from respective recurrent parents.

11.1 PCR Conditions

PCR shall be set up in 10 µl volume using 10 ng template DNA, 10 µM of each of the primers and 1 U of DNA Taq polymerase master mix (other reagents such as dNTPs, buffer and $MgCl_2$ as specified in Table 1 may be used in place of Master mix). The PCR program/amplification conditions as given in Table 3 shall be followed.

12 QUANTIFICATION OF ADULTERATION

12.1 General

The factors affecting quantification of adulteration in Basmati rice samples are:

a) Equivalent contribution of DNA from the grain mixtures, and

b) Faithful representation of the samples.

Therefore, adulterant is quantified based on the relative quantities of the amplified allelic products at a common locus between competing DNA templates (those of the Basmati and the adulterant) in the PCR mixture (*see* Fig. 1).

Table 7 Gene Based Markers for the Distinction of Resistant Basmati Varieties from their Respective Recurrent Parents

(*Clause* 11)

Sl No.	Variety	Recurrent Parent	Genes	Specific Traits	Marker	Primer Sequence		Product Size (in Base Pairs)
						Forward (5' to 3')	Reverse (5' to 3')	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
i)	Pusa Basmati 1718	Pusa Basmati 1121	Xa13	Resistance to bacterial blight	xa13-prom	GGCCATGGCTCAGTGTTTAT	GAGCTCCAGCTCTCCAAATG	500, 270
			Xa21	Resistance to bacterial blight	pTA248	AGACGCGGAAGGGTGGTTCCCGGA	AGACGCGGTAATCGAAGATGAAA	950, 650
ii)	Pusa Basmati 1728	Pusa Basmati 6	Xa13	Resistance to bacterial blight	xa13-prom	GGCCATGGCTCAGTGTTTAT	GAGCTCCAGCTCTCCAAATG	500, 270
			Xa21	Resistance to bacterial blight	pTA248	AGACGCGGAAGGGTGGTTCCCGGA	AGACGCGGTAATCGAAGATGAAA	950, 650
iii)	Pusa Basmati 1637	Pusa Basmati 1	Pi9	Resistance to Blast	AP5659-5	AGCTCTTCTGAAAAGTGCAGTG	TGATGACTTCCAAACGGTAG	290, 300
iv)	Pusa Basmati 1609	Pusa Sugandh 5	Pi54	Resistance to Blast	RM206	CAATCTCCAAAGTTTTCAGG	GCTTCAATCACTGCTAGACC	140, 180

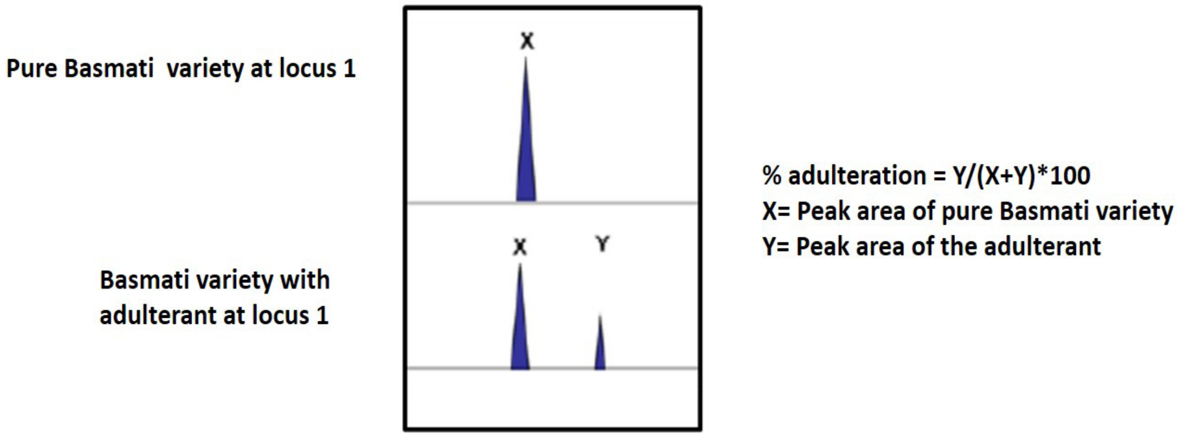

FIG. 1 QUANTIFICATION OF THE ADULTERANT

12.2 Adulteration is expressed as a percent fraction:

$$\text{Adulteration} = \frac{Y \times 100}{X + 100}$$

Where,

Y = relative fluorescent unit (rfu) of the adulterant; and

X = rfu of the Basmati variety.

NOTE — Since the legal ceiling for adventitious adulteration even for the lowest grade Basmati has been proposed at 7 percent, accuracy of estimation of adulteration beyond 15 percent is rendered redundant.

ANNEX A

(*Informative*)

Table A: Primary and Ancillary Quality Characteristics of Basmati Rice as Defined in the Memorandum of Ministry of Agriculture, Government of India (No. 17-12/2007-SD.IV dated 29th May 2008)

Sl No.	Parameters	Value
Primary		
1	Minimum average precooked milled rice length (mm)	6.61
2	Average precooked milled rice breadth (mm)	≤ 2.00
3	Minimum length/breadth ratio of precooked milled rice (L/B ratio)	3.50
4	Minimum average cooked rice length (mm)	12.00
5	Minimum cooked rice length/precooked rice length ratio or Minimum elongation ratio	1.70
6	Average volume expansion ratio	>3.5
7	Aroma	Present
8	Texture of cooked grain for high integrity (without bursting the surface), non-stickiness, tenderness, good taste and good mouth feel	Present
Ancillary		
1	Amylose content range	20-25 percent
2	Alkali spreading value range (ASV)	4.0-7.0
3	Minimum brown rice recover (percent)	76 percent
4	Minimum milled rice recovery (percent)	65 percent
5	Minimum head rice recovery (percent)	45 percent

Table B: Basmati Growing States of India Under GI

Sl No.	States	Districts
1	Punjab	Entire State
2	Haryana	Entire State
3	Delhi	Entire State
4	Himachal Pradesh	Entire State
5	Uttarakhand	Entire State
6	Jammu & Kashmir	Jammu & Kathua
7	Uttar Pradesh	Agra, Aligarh, Badaun, Baghpat, Bareilly, Bijnore, Blandshahr, Etah, Etawah, Farrukhabad, Firozabad, Gautam Budh Nagar, Ghaziabad, Hathras, J.P. Nagar, Kannuj, Mainpuri, Mathura, Meerut, Moradabad, Muzaffarnagar, Oraiya, Pilibhit, Rampur, Sahajahanpur, Saharanpur.

Table C: Details of the 32 Basmati Rice varieties Notified under *Seeds Act*, 1966

Sl. No.	Variety	IET No	Notification No	Name of Institution/ Univ.	Parentage
1	Basmati 217	-	4045– 24.09.1969 361 (E) – 30.06.1973	Punjab Agricultural University, Ludhiana	-
2	Basmati- 370	-	361(E)– 30.06.1973 786 – 02.02.1976	Rice Farm, Kalashah Kaku (now in Pakistan)	Selection from local agro commercial group of Basmati at Govt. Farm, Kala Shah Kaku in Shekhpura district in pre-partition Punjab

Table B (*Continued*)

Sl. No.	Variety	IET No	Notification No	Name of Institution/ Univ.	Parentage
3	Dehradun Basmati (Type-3)	-	13–9.12.1978	Rice Research Station, Nagina, U.P.	Selection (Type-3)
4	Ranbir Basmati	IET-11348	1(E)-01.01.1996	Rice Research Station, R.S. Pura, Jammu	A selection from Basmati 370
5	Basmati- 386	IET14710	647(E)–09.09.1997	Punjab Agricultural University, Ludhiana	Pure line selection
6	Taraori Basmati (HBC-19)	-	1(E)– 01.01.1996	CCSHAU, Rice Research Station, Kaul, Dst. Kaithal, Haryana	Selection from local Basmati
7	Punjab Basmati-1	-	4045– 24.09.1969 361 (E) – 30.06.1973	Punjab Agricultural University, Ludhiana	-
8	CSR-30 (Yamini)	IET-14720	1134(E)– 25.11.2001 2126(E)– 10.09.2012	The Central soil Salanity Ressearch Institute, Karnal, Haryana	BR-4-10/Pak Basmati
9	Mahi Sugandha	IET-12601	408(E)- 04.05.1995	Rice Research Station, Banswara, Rajasthan	BK79/Basmati 370
10	Kasturi	IET-8580	615(E)- 06.11.1989	Directorate of Rice Research, Rajendra Nagar, Hyderabad, A.P	Basmati370/CR88-17-1-5
11	Haryana Basmati 1	IET-10367	793(E)- 22.11.1991	CCSHAU, Rice Research Station, Kaul Dst. Kaithal, Haryana	Sona/Basmati 370
12	Punjab Basmati-2	IET 18973	1708(E)– 26.07.2012	Punjab Agricultural University, Ludhiana	Basmati 386/Super Basmati
13	Pusa Basmati-1	IET-10364	615(E)– 06.11.1989	The Indian Agricultural Research Institute, New Delhi	Pusa 150/Karnal local
14	Pusa-1460 (Improved PB1)	IET-18990	1178(E)– 20.07.2007	The Indian Agricultural Research Institute, New Delhi	Pusa Basmati 1//Pusa Basmati 1/IRBB55
15	Pusa Basmati-1401 (PB-6)	IET-18005	733(E)– 01.04.2010	The Indian Agricultural Research Institute, New Delhi	Pusa Basmati 1/Pusa 1121-92-8-2-7-1
16	Pusa Basmati- 1121	IET-18004	1566(E)– 05.11.2005 2547(E)- 29.10.2008	The Indian Agricultural Research Institute, New Delhi	Pusa 614-1-2/Pusa 614-2-4-3
17	Pusa Basmati- 1509	IET-21960	2817(E)– 19.09.2013	The Indian Agricultural Research Institute, New Delhi	Pusa 1301/Pusa 1121
18	Vallabh Basmati-21	IET-19493	2817 (E)– 19.09.2013	Sardar Vallabh Bhai University of Agriculture and Technology, Modipuram	Khalsa 7/Pusa 1121)/Type-3
19	Vallabh Basmati- 22	IET-19492	2187 (E)– 27.08.2009	Sardar Vallabh Bhai University of Agriculture and Technology, Modipuram	-
20	Vallabh Basmati-23	IET-19491	268 (E) – 28.01.2015	Sardar Vallabh Bhai University of Agriculture and Technology, Modipuram	Pusa 1121/Type-3
21	Vallabh Basmati-24	IET-20827	268 (E) – 28.01.2015	Sardar Vallabh Bhai University of Agriculture and Technology, Modipuram	Pusa Basmati-1/Type3

Table B (*Concluded*)

Sl. No.	Variety	IET No	Notification No	Name of Institution/ Univ.	Parentage
22	Malviya Basmati	IET-21669	2817 (E)– 19.09.2013	Banaras Hindu University, Varanasi, UP	HBR 92/Pusa Basmati/ Kasturi
23	Basmati 564	IET-17269	268 (E) – 28.01.2015	Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha, Jammu	-
24	Pusa Basmati- 1609	IET-22778	2680 (E) - 1.10.2015	The Indian Agricultural Research Institute, New Delhi	Pusa 1602/Pusa 1603
25	Pant Basmati-1	IET-21665	112 (E) – 12.01.2015	GBPUA&T, Pant Nagar	-
26	Pant Basmati-2	IET-21953	112 (E) – 12.01.2015	GBPUA&T, Pant Nagar	-
27	Punjab Basmati-3	IET21966	3540 (E)– 22.11.2016	Punjab Agricultural University, Ludhiana	Bas386/IET 17948// Bas 386*2
28	Pusa Basmati 1718	IET 24565	2805 (E) – 25.8.2017	The Indian Agricultural Research Institute, New Delhi	PB 1121/SPS 97//PB 1121*3
29	Pusa Basmati 1728	IET 24573	3540 (E)– 22.11.2016	The Indian Agricultural Research Institute, New Delhi	Pusa Basmati 6/Pusa 1460//Pusa Basmati 6*3
30	Pusa Basmati 1637	IET 24570	3540 (E)– 22.11.2016	The Indian Agricultural Research Institute, New Delhi	Pusa Basmai 1/IRBL 9-W//Pusa Basmai 1*3
31	Punjab Basmati-4 (RYT 3404)	IET-25399	1379(E)– 27.3.2018	Punjab Agricultural University, Ludhiana	-
32	Punjab Basmati-5 (RYT 3432)	IET 26153	1379(E)– 27.3.2018	Punjab Agricultural University, Ludhiana	-

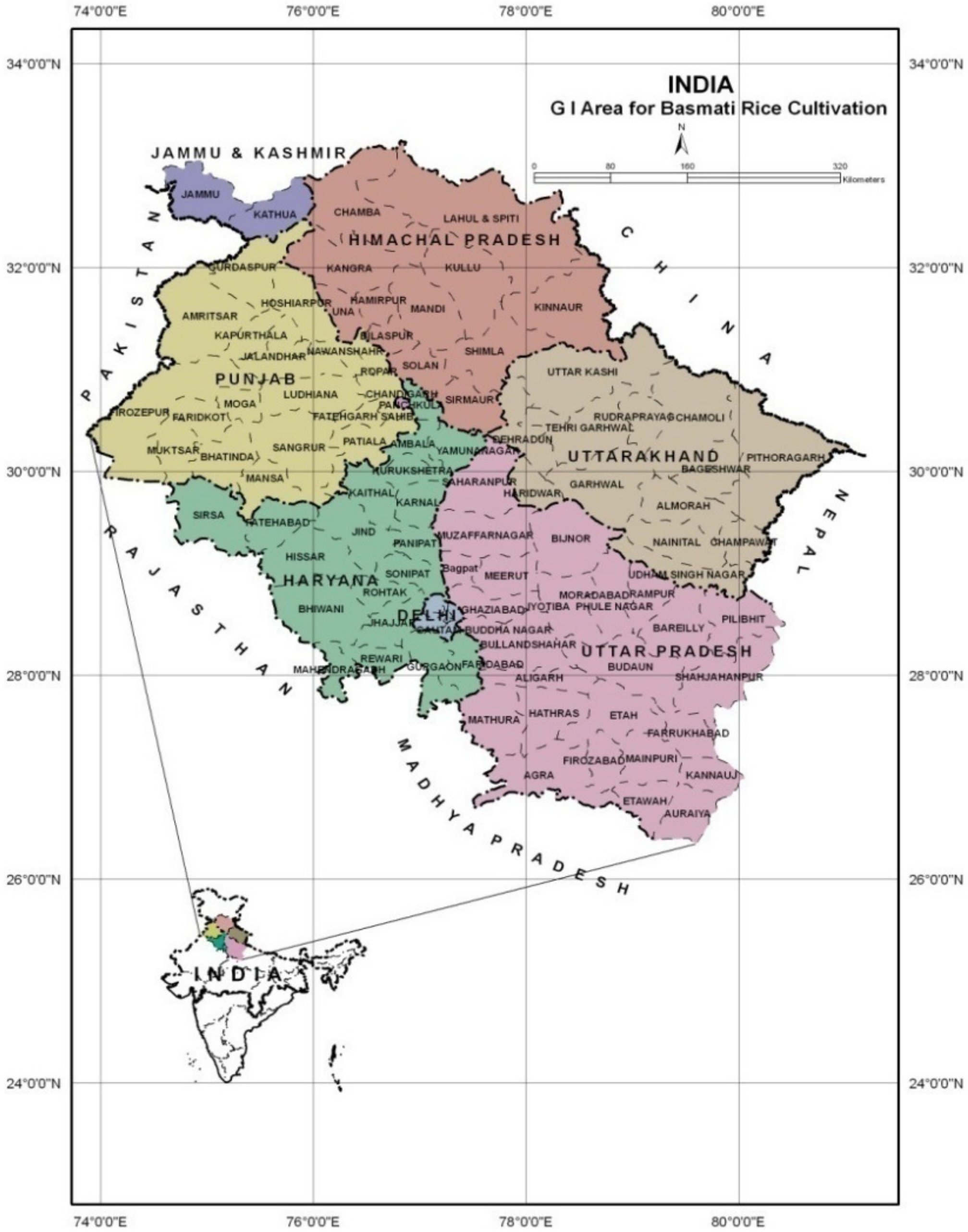

FIG. A-1 GI AREA OF BASMATI RICE CULTIVATION

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